

$\delta^{15}\text{N}$ analyses of butterfly wings and bodies suggest minimal nitrogen absorption in carrion and dung puddling butterflies (Lepidoptera: Nymphalidae)

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Abstract. Nitrogenous compounds in the adult diet can play an important role in the nutritional ecology and life history evolution of butterflies. We compared the concentration of $\delta^{15}\text{N}$ in fruit-feeding butterflies that feed on faeces and carrion (puddling) versus those that do not feed on these substrates, and used the difference between $\delta^{15}\text{N}$ in bodies versus wings as a measure of the amount of nitrogen derived from animals. Differences between the sexes and between the feeding guilds were small, suggesting that minimal amounts of nitrogen were absorbed from these resources. Males, the sex that does most of the puddling, had higher $\delta^{15}\text{N}$, but this occurred in both carrion feeders and non carrion feeders. This probably reflects differences in metabolic activity of caterpillars. It appears that caterpillar metabolism and metamorphosis results in significant secondary $\delta^{15}\text{N}$ enrichment and differences among the sexes, and, together with host-plant range and quality, in within species variation in $\delta^{15}\text{N}$ values.

Key words: fruit-feeding, host-plant, nitrogen metabolism, sexual differences, tropical forest, Uganda

INTRODUCTION

The commonly observed puddling behavior (feeding on mud, dung or carrion) of especially male butterflies is often interpreted as foraging for sodium (Arms *et al.*, 1974; Molleman *et al.*, 2005; Norris, 1936; Pivnick & Mcneil, 1987; Smedley & Eisner, 1995, 1996). However, carrion and faeces from predators also contain proteins and amino acids and some butterfly species show preferences for these components in the context of nectar feeding (Erhardt & Rusterholz, 1998; Mevi-Schutz & Erhardt, 2003) and puddling (Beck *et al.*, 1999; Boggs & Dau, 2004). Usage of adult diet derived nitrogen for reproduction may provide opportunities for life history evolution, including extension of life span (Dunlap-Pianka *et al.*, 1977). Males mostly puddle and males can transfer nutrients to the female in the spermatophore. Therefore, nutrients collected by the male can be used in female metabolism and reproduction (Oberhauser, 1989; Wiklund *et al.*, 1993).

$\delta^{15}\text{N}$ increases by about 3-5 ‰ per trophic level (Peterson & Fry, 1987). Butterfly wings are fully developed at eclosion and besides small amounts of

haemolymph, will thus mainly reflect the nitrogen source of larvae, whereas the bodies will reflect that of caterpillars as well as subsequent adult food. Thus, a male butterfly that fed on the remains of an insectivore in the faeces of a civet cat may be utilizing nitrogen that is 9-15 ‰ higher in $\delta^{15}\text{N}$ than its own wings. Differences in $\delta^{15}\text{N}$ between body and wings of an individual may thus indicate to which extent adults utilize animal derived nitrogen.

Plants vary in their $\delta^{15}\text{N}$ depending on nitrogen sources (e.g. mycorrhiza or bacterial fixation (Hobson, 1999)). In addition, different food plants may cause different degrees of metabolic fractionation of $\delta^{15}\text{N}$ in herbivores. Thus intra-specific variation in $\delta^{15}\text{N}$ indicates the range of food-plants used. For example, Webb *et al.* (1998) noted that locusts fed on nutrient poor diet were enriched by 7.8 ‰ in $\delta^{15}\text{N}$, whereas they were only enriched by 3.1 ‰ on a nutrient rich diet. In addition, variations in butterfly $\delta^{15}\text{N}$ content may reflect metabolic differences, especially among the sexes.

MATERIALS AND METHODS

Butterflies were collected from fruit-baited traps at Makerere University Biological Field Station in Kibale Forest National Park, Western Uganda (0° 35'

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Table 1. $\delta^{15}\text{N}$ values (‰) of bodies and wings and thorax width (mm) of fruit-feeding butterflies from Kibale National Park, Uganda

Species	Male					Female						
	thorax width	N	body mean	range	wing mean	range	thorax width	N	body mean	range	wing mean	range
Faeces and carrion feeding:												
<i>Charaxes fulvescens</i>	7.2	11	6.28	(2.52-8.15)	6.16	(2.58-8.42)	7.5	7	5.73	(2.37-8.76)	5.99	(2.51-8.65)
<i>Charaxes bipunctatus</i>	9.3	6	9.44	(6.52-10.87)	9.67	(7.04-10.93)	9.4	4	8.50	(6.92-10.18)	8.22	(7.10-9.9)
<i>Charaxes numenes</i>	8.7	2	7.07	(6.86-7.29)	7.47	(7.1-7.84)	8.6					
<i>Charaxes tiridates</i>	9.4	1	7.88		8.83		10.0	1	5.65		5.92	
<i>Harma theobene</i>	5.0	1	5.56		5.34		4.7					
Not faeces and carrion feeding:												
<i>Euphaedra alacris</i>	6.3	10	8.54	(6.33-9.98)	8.32	(6.54-9.41)	6.4	9	7.51	(5.73-9.03)	7.58	(6.15-9.65)
<i>Euphaedra medon</i>	5.8	10	7.02	(3.65-9.75)	7.05	(3.74-9.25)	5.7	8	7.37	(4.15-9.54)	6.56	(4.19-10.09)
<i>Euphaedra harpalyce</i>	6.5	7	5.65	(4.16-7.18)	6.16	(4.38-7.21)	6.7	3	6.16	(5.82-8.44)	7.01	(5.26-8.27)

N 30° 20' E) in July 2001. The field station borders selectively logged moist evergreen forest at an altitude of around 1500 m and is therefore classified as a transition towards montane forest. The mean maximum temperature is 23.8°C and the mean annual rainfall is 1749 mm and is bimodal in distribution (Chapman *et al.*, 2005).

The carrion feeding species included were; *Charaxes fulvescens* Aurivillius, 1891, *C. bipunctatus* Rothschild, 1894, *C. numenes* (Hewitson, 1859), *C. tiridates* (Cramer, 1777) and *Harma theobene* Doubleday 1848, the non-carrion feeding species were *Euphaedra alacris* Hecq, 1979, *E. medon* Linnaeus 1763 and *E. harpalyce* (Cramer, 1777). Specimens showing signs of wear of the wings were selected to focus on older individuals that had had opportunity to puddle. The feeding habits and thorax widths were documented using traps with rotting fish or civet dung (Molleman *et al.*, 2005). Butterflies were dried within glassine envelopes and thereafter bodies and wings were mill-ground separately. A sample from body and from wing was thereafter analyzed on the University of Cape Town mass spectrometer (Finnigan Mat 252) after combustion in an automated Carlo Elba device, using Merck gel standards.

RESULTS

All results are summarized in Table 1. Neither male nor female bodies were $\delta^{15}\text{N}$ enriched compared to

wings. The relationship between body and wing $\delta^{15}\text{N}$ within individuals is both very strong and close to a slope of unity: slope for male and female non-carrion feeding wing versus body is 0.91 ($r=0.96$, $n=46$) and for carrion eaters it is 0.99 ($r=0.92$, $n=33$). Within individual differences in body and wing $\delta^{15}\text{N}$ between the sexes and feeding guilds are small. Males may have a higher $\delta^{15}\text{N}$ in both bodies and wings, than do females in both carrion and non-carrion feeding butterflies (e.g. for bodies Mann-Whitney U: $p<0.20$, $n=77$). Within each species, there was a wide range of $\delta^{15}\text{N}$ values. Larger species may have higher $\delta^{15}\text{N}$ values, at least among males (correlation between thorax width and average $\delta^{15}\text{N}$ in body: $N=8$, $r=0.6$, $p=0.11$).

DISCUSSION

On the basis of our results it seems unlikely that the species that feed on faeces and carrion obtain significant amounts of nitrogen from this source, because the difference between wings and bodies is small in both sexes and is not different amongst the two feeding guilds. However, we did not measure $\delta^{15}\text{N}$ of the spermatophore or eggs of carrion feeders versus non carrion feeders, and animal derived nitrogen may be incorporated into the spermatophore and thereafter transferred to the eggs. In addition, particular individuals of species that are known to feed on carrion and dung may not (or rarely) have fed on these substrates, so that little or no animal derived

nitrogen could be used in adult metabolism.

That male bodies may be enriched compared to females is not due to supplementary adult nitrogen feeding, because their wings are also relatively enriched. The differences between the sexes likely reflects sexual metabolic differences among caterpillars, as was shown by Pivnick and McNeil (1987) for the puddling butterfly *Thymelicus lineola*: male larvae consume about 85% of the foliage consumed by females, yet on adult emergence, males have a dry weight of only 48% of the females. However, at emergence males contain 82% of the total body sodium of females and the concentration is twice that in females. This suggests that caterpillars exhibit compensatory feeding for sodium. These results indicate that to understand puddling and its relationships to nutrient budgeting, an approach incorporating both larval and adult feeding will be necessary.

A surprising result was the large intra-specific variation in wing and body $\delta^{15}\text{N}$ as a range of more than 5 ‰ occurs in several species. This would suggest broad host-plant ranges. However, for some of the species included, few host-plants have been recorded (Molleman & Hecq, 2005: *E. medon* 1, *E. alacris* 2, *E. harpalyce* 2; F. Molleman unpublished data, Larsen (1991): *C. fulvescens* 1-2, *C. bipunctatus* 1), while *C. tiridates* and *C. numenes* caterpillars have been found on a variety of plant genera (Larsen, 1991) and *Harma theoben* was found on one plant species in Kibale Forest (F. Molleman, unpublished data) but has been collected from some others elsewhere (Larsen, 1991). For the host-plant of *Charaxes fulvescens*, *Allophylus* species, we obtained a mean $\delta^{15}\text{N}$ of 1.74 ‰ for two bulked leaf samples, considerably lower than the 8.15 ‰ maximum we obtained for this species. These results suggest that caterpillar metabolism and metamorphosis result in significant secondary $\delta^{15}\text{N}$ enrichment.

CONCLUSIONS

This study illustrates the usefulness of stable isotope analyses for elucidating variation in metabolism and nutritional ecology among insects. We found no evidence that adult carrion or fruit-feeding butterflies metabolize animal derived nitrogen. It appears that caterpillar metabolism and metamorphosis results in significant secondary $\delta^{15}\text{N}$ enrichment and differences among the sexes, and, together with host-plant range and quality, in within species variation in $\delta^{15}\text{N}$ values.

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